

*February 21, 1889.*

Professor G. G. STOKES, D.C.L., President, in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Papers were read :—

I. "The Influence of Bile on the Digestion of Starch. I.—Its Influence on Pancreatic Digestion in the Pig." By SIDNEY MARTIN, M.D. (Lond.), B.Sc., British Medical Association Scholar, and Assistant Physician to the City of London Hospital for Diseases of the Chest, Victoria Park, and DAWSON WILLIAMS, M.D. (Lond.), Assistant Physician to the East London Hospital for Children, Shadwell. Communicated by E. A. SCHÄFER, F.R.S. (from the Physiological Laboratory, University College, London). Received February 1, 1889.

The object of the research is to ascertain what influence, if any, the presence of bile or its constituents has on the progress and result of pancreatic digestion; it includes the investigation of any such influence on the amylolytic, the proteolytic, and the emulsive ferments. The present communication deals only with the first named; our experiments have been done chiefly with the pancreas and bile of the pig, but another series in which these secretions in other animals are being examined is in progress; the effect of the presence of bile on all amylolytic digestion, *ex. gr.*, that of saliva and that of vegetable diastase, is a subject which also seems to be worthy of investigation, and is now receiving our attention. In the present communication we detail the result of our experiments with the bile and pancreatic amylolytic ferment of one animal only—the pig.

The fluid to be digested has been made by boiling pure starch in distilled water and carefully neutralising if necessary. Starch 2 grams, water 100 c.c., has been found a convenient strength. Pig's bile has been used either in the fresh state or after careful drying at a temperature not exceeding 27° C. In the later form it was found more convenient for preserving and for manipulation, as it could be accurately weighed. Glycerine extract of fresh pig's pancreas, and

a commercial pancreatin made from pig's pancreas, and ascertained to be rich in the amylolytic ferment, have been used.

Our earliest experiments indicated that bile had a very notable influence on the pancreatic digestion of starch; it caused a rapid disappearance of the blue reaction of starch with iodine.

*Experiment A.*—Five tubes, *a*, *b*, *c*, *d*, *e*, each containing 50 c.c. of the starch mixture (2 per cent.). With *c* 2·0 c.c. and with *d* and *e* 8·0 c.c. fresh pig's bile were thoroughly mixed. Equal quantities of glycerine extract of pig's pancreas were then simultaneously added to *b*, *c*, and *d*, and all five tubes were placed in a water-bath at 33° C. The colour reaction of solution of iodine with the two control tubes—*a* which contained the starch mixture alone, and *e* which contained the starch mixture and bile (8 c.c.)—remained unaltered throughout the experiment. The changed colour reaction in the other tubes was watched by mixing a drop of the mixture with iodine solution on a white porcelain plate. The blue reaction in *d* rapidly disappeared, being replaced in less than one minute by a purple and in two minutes by a red colour; the red colour became gradually fainter and had entirely disappeared in ten minutes. In *b* and *c* the blue reaction disappeared more slowly, a purple colour being still obtained at the end of ten minutes; no difference was perceptible in this respect between *b* and *c*, a fact which indicates that the amount of bile present must exceed the proportion added to *c* before any accelerating influence was noticeable.

By using weighed quantities of the dried bile it was proved that a larger proportion of bile caused the blue reaction with iodine to disappear more rapidly than a small proportion.

*Experiment B.*—Four vessels, *a*, *b*, *d*, *e*, containing the starch mixture 2 per cent. To *b* 0·6 per cent. dried pig's bile, to *d* and *e* 3 per cent. dried pig's bile were added and dissolved; to *a*, *b*, and *d* equal quantities of glycerine extract of pig's pancreas were added, and all the vessels were placed in a water-bath at 33° C.; *d* ceased to give any colour reaction with iodine solution in five minutes; at the same moment the reaction given by *b* was reddish-purple, and by *a* purple; *e* remained unchanged.

This increase of rapidity with increasing proportion of bile was found to hold up to 4 per cent. of dried bile (equivalent probably to at least 30 per cent. fresh bile). Beyond this percentage we have not made experiments; a larger proportion of bile rendered the mixture very thick and interfered with the colour reaction.

It was also ascertained that the amount of sugar, estimated as dextrose, formed under the conditions of Experiments A and B, was greater when bile was present, and increased when the proportion of bile was increased.

*Experiment C.*—Four vessels, *a*, *b*, *c*, *d*, containing the starch

mixture 2 per cent. To *b* 0·6 per cent. dried pig's bile, and to *c* and *d* 2 per cent. dried pig's bile were added and dissolved; to *a*, *b*, and *c* equal quantities of glycerine extract of pig's pancreas were added and all the vessels were placed in a water-bath at 34° C. The colour reaction with iodine given by *d* was unchanged throughout, but *a*, *b*, and *c* gave a varying colour reaction, and changing most rapidly with *c* and least rapidly with *a*. After remaining in the water-bath for eight minutes the vessels were taken out and their contents boiled, to destroy the ferment, and the amount of dextrose estimated by Fehling's method; *a* contained 0·45 per cent., *b* 0·59 per cent., and *c* 0·74 per cent.

A large number of experiments were performed of which the above are quoted as examples, and the conclusion to which we were led was that digestion of starch by extract of pig's pancreas was hastened in the presence of pig's bile. We next sought to ascertain (1) whether this was a property of the bile solids as a whole, or of one or other constituent; and (2) the nature of this hastening action, whether, that is to say, the bile only hastened the transformation of starch into dextrin, or whether there were also constant increase in the amount of sugar formed.

*Firstly*, as to whether the effect is to be ascribed to the action of any one constituent of the bile. Pig's bile contains bile salts (chiefly hyoglycocholate of sodium\*), bile pigment, cholesterol, soaps, and salts together with mucin. We found that an extract of dried bile made with absolute alcohol retained the power of hastening pancreatic digestion of starch, and finally that it was also possessed by the bile salts. It was found in this case also that the amount of sugar estimated as dextrose was greater as the proportion of bile salts added to the mixture was increased up to 2 per cent., beyond which our experiments have not gone. Thus in one experiment the amount of sugar found after half an hour's digestion (*a*) in a mixture to which 0·6 per cent. of bile salts had been added = 1·03 per cent.; (*b*) in a mixture to which 2·0 per cent. of bile salts had been added = 1·25 per cent.; and (*c*) in a mixture to which no bile salts had been added 1·0 per cent.; a large amount of starch mixture was used in this experiment and 0·8 per cent. pancreatin added.

*Secondly*, as to the nature of the process, whether the bile hastened the transformation of starch into dextrin, or whether there was also an increase in the amount of sugar; this was found to be a somewhat difficult question to solve. The quantitative estimation of a mixture of starch, dextrin, and sugar, or of dextrin and sugar was found to present many difficulties. The amount of sugar was readily estimated

\* Jolin ('Zeits. f. Physiol. Chemie,' vol. 11, p. 417) describes  $\alpha$ - and  $\beta$ -hyoglycocholate.

as dextrose by Fehling's method, but we are unacquainted with any reagent which will effect the separation of dextrin from starch; they can both, however, be precipitated by absolute alcohol. We have made a quantitative estimation of the relative amounts of starch, dextrin, and sugar by the following method: two equal portions of the starch mixture, 2 per cent., were digested with equal quantities of dried pig's pancreatin,\* rich in amylopsin, a certain proportion of bile salts (made from pig's bile) having been previously added to one. Digestion was allowed to proceed in the incubator until the reaction of starch with a solution of iodine had completely, or almost completely disappeared from the vessel to which bile salts had been added. Both mixtures were then rapidly boiled to stop the action. The digested mixture was then poured into a dialyser (made of German sausage paper) and dialysed in running water for four or five days, thymol being added to prevent decomposition (which did not occur); the dextrin, sugar, and most of the salts were thus dialysed away, and the total residue (starch) was estimated by evaporating the dialysed liquid to small bulk and filtering into alcohol. The precipitate was caught on a filter, dried at 100° to 110° C., and weighed. The residue of undigested starch was thus estimated. The proportional amounts of sugar and dextrin were estimated by dialysing the liquids digested under the same conditions as those just described, in distilled water for four days, decomposition being prevented by the daily addition of thymol. Equal quantities of the two dialysates, the one containing sugar and dextrin, the other sugar, dextrin, and bile salts, were evaporated to small bulk, the sugar estimated as dextrose by Fehling's solution, the dextrin by precipitating a measured quantity of each concentrated liquid by absolute alcohol, washing with absolute alcohol to remove bile salts, drying at 100° to 110° C., and weighing.

The results are shown in the following experiments:—

*Experiment D.*—To one of two flasks containing 200 c.c. of the starch mixture (2 per cent.) 0·6 per cent. bile salts was added; 0·8 per cent. pancreatin was then added to both flasks and the mixture digested at 38° C. for two minutes. The flask containing bile salts then gave no reaction with iodine solution, while that which contained pancreatin alone gave a purple reaction. Both fluids were then dialysed in cold distilled water for four days, decomposition being prevented by the daily addition of thymol. Both dialysates, which were faintly acid and contained no starch, were then evaporated to small bulk, and each divided into two parts for the estimation of sugar and dextrin respectively. The former was estimated as dextrose by Fehling's process, the latter by precipitating under absolute alcohol, filtering, drying at 100—110° C., and weighing. The result was:—

\* Prepared by Messrs. Savory and Moore.

	Dextrin.	Sugar.
Fluid to which bile salts had been added as well as pancreatin.....	0·30 gram.	1·315 gram.
Fluid to which pancreatin only was added.....	0·24168 ,,	1·042245 ,,

The addition of bile salts therefore had increased the production of sugar in the proportion 5:4, and that of dextrin in like proportion.

*Experiment E.*—This experiment was conducted with the same proportion of each ingredient and in the same manner, with the exception that the fluids were dialysed in a stream of (tap) water; the total residue, after evaporation and treatment with absolute alcohol in the manner previously described, was estimated by drying and weighing. The residue in the fluid containing bile salts weighed 0·314 gram, in the fluid to which pancreatin alone was added, it weighed 0·517 gram. These residues contained starch and a trace of peptone, but no bile salts nor sugar.

Our conclusions may thus be briefly stated:—The effect of fresh and dried bile in hastening the pancreatic digestion of starch in the pig is due to the bile salts; these salts possess the power of increasing the amount not only of dextrin, but of sugar estimated as dextrose.

The authors are not at present in a position to explain this influence of bile salts; the pancreatic solution of starch proceeds more rapidly at first in laboratory experiments, and the retardation after a short interval is very marked. It is possible that the bile salts may favour its continuance by entering into combination with the bodies which have this retarding effect.

## II. "The Innervation of the Renal Blood-vessels." By J. ROSE BRADFORD, M.B., D.Sc., George Henry Lewes Student. Communicated by E. A. SCHÄFER, F.R.S. (from the Physiological Laboratory of University College, London). Received February 1, 1889.

The following work was undertaken in order to map out the origin, course, and nature of the renal nerves more accurately than had hitherto been attempted. It was considered (more especially in the light of Gaskell's well-known work on the sympathetic) important to decide whether the renal and other abdominal vascular nerves were of two kinds, *i.e.*, vaso-constrictor and vaso-dilator, or whether the latter nerves could not be demonstrated to exist. This research was carried out exclusively on the dog, inasmuch as this was the animal used by Gaskell in his work.